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Editorial

Low dose effects and non-monotonic dose responses for endocrine active chemicals: Science to practice workshop: Workshop summary[☆]



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HIGHLIGHTS

- Report of workshop on low dose and non-monotonic effects of endocrine disruptors.
- Need for research on low dose effects and non-monotonic dose responses to EDCs.
- No consensus on importance of non-monotonic responses to risk assessment.
- Changes needed to risk assessments to accommodate EDC effects.
- More workshops and improved communication between relevant parties.

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ABSTRACT

A workshop was held in Berlin September 12–14th 2012 to assess the state of the science of the data supporting low dose effects and non-monotonic dose responses (“low dose hypothesis”) for chemicals with endocrine activity (endocrine disrupting chemicals or EDCs). This workshop consisted of lectures to present the current state of the science of EDC action and also the risk assessment process. These lectures were followed by breakout sessions to integrate scientists from various backgrounds to discuss in an open and unbiased manner the data supporting the “low dose hypothesis”. While no consensus was reached the robust discussions were helpful to inform both basic scientists and risk assessors on all the issues. There were a number of important ideas developed to help continue the discussion and improve communication over the next few years.

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1. Introduction

There are continuing discussions in Europe and the United States to identify and develop the best methods to translate scientific findings to human health risk assessment. Risk assessment

processes, used by regulatory agencies around the world, have been developed based on the principles of toxicology where it is generally assumed that the response of an organism to a toxicant increases with increasing level and duration of exposure (known as a monotonic dose response). Moreover for many chemicals a

Abbreviations: ANSES, French Agency for Food, Environmental and Occupational Health & Safety; BfR, Bundesinstitut für Risikobewertung, German Federal Institute for Risk Assessment; BPA, bisphenol A; EAC, endocrine active compound; EDC, endocrine disrupting chemical; EFSA, European Food Safety Authority; EPA, US Environmental Protection Agency; FDA, US Food and Drug Administration; GLP, Good Laboratory Practices; NGO, non-governmental organization; NIEHS, National Institutes of Environmental Health Sciences; NMDR, non-monotonic dose response; NMDRC, non-monotonic dose response curve; NOAEL, no observed adverse effect level; NTP, US National Toxicology Program; OECD, Organization for Economic Cooperation and Development.

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threshold approach is applied which assumes that there is no adverse effect below a certain exposure level. However there is a class of toxicants, endocrine disrupting chemicals (EDCs), for which there is evidence that they do not obey the principles of toxicology. Thus there are data showing effects at doses below apparent no effect levels in toxicity studies conducted according to current standard protocols. In addition, there are data showing that EDCs in some cases show non-monotonic dose responses (NMDRs). In these cases extrapolation from effects observed at high doses to human/environmental exposure levels may not be applicable. This so-called ‘low dose hypothesis’ challenges the traditional dose–response paradigm in toxicology and has been received with skepticism and caution by some scientists including many risk assessment practitioners. This topic is of special interest now because of the need to develop criteria for the identification and assessment of EDCs for application under various chemical control regulations in the European Union.

Over the past decade there have been several meetings addressing the “low dose” paradigm and its implications for risk assessment. The first formal assessment of the effects of chemicals at doses lower than those traditionally tested was held at the National Institute of Environmental Health Sciences (NIEHS) in collaboration with the US Environmental Protection Agency (EPA) in 2001 (Melnick et al., 2002). This scientific peer review of the data provided a “rigorous, open, transparent, and objective evaluation of the scientific evidence showing the presence or absence of low-dose effects of endocrine disrupting agents. . .”. The workshop verified low dose effects for four EDCs (diethylstilbestrol, genistein, methoxychlor, and nonylphenol) and estradiol. The workshop report noted that “the findings of the panel indicate that the current testing paradigm used for assessments of reproductive and developmental toxicity should be revisited to see whether changes are needed regarding dose selection, animal-model selection, age when animals are evaluated, and the end points being measured following exposure to endocrine-active agents.” In the following years there were reviews focused on “low dose” effects of bisphenol A (BPA) (vom Saal and Hughes, 2005; vom Saal et al., 2007) but no institutional attempts to analyze or examine the wider low dose literature.

In 2009, the German Federal Institute for Risk Assessment (BfR) held a workshop to establish assessment and decision criteria in human risk assessment for substances with potential endocrine disrupting properties focusing on active substances in plant protection products (Federal Institute for Risk Assessment (BfR), 2009). While this workshop was not focused on low dose effects, one point of discussion was whether effects occur at doses below those normally tested and if NMDRs exist for EDCs. Several recommendations were made:

1. Robust evidence of low dose effects of endocrine disrupting substances was considered to be important to be established before regulatory action might be taken. This evidence should include reproducibility of effects with the same compound in different studies.
2. Funding of international projects for the validation of methods and the development of new methodology to assess low dose effects as well as the development of a literature search on evidence for potential low dose effects of substances with endocrine disrupting properties were recommended.
3. The development of workshops on low dose issues was considered to be of major relevance.

Responding to BfR’s meeting conclusions, a group of scientists developed a comprehensive review of the low dose and NMDR literature (Vandenberg et al., 2012). The authors concluded that low dose effects and NMDRs are to be expected for chemicals with

endocrine disrupting activity and that these responses may occur frequently enough to be a concern. The review focused in part on the evidence of associations between current human exposures to various chemicals and specific diseases and in part on the data showing that these observations are supported by mechanistic *in vitro* and animal studies.

The Vandenberg et al. review stimulated the development of several workshops on the topic of low dose effects and NMDR. For instance, shortly after its publication The Pew Charitable Trusts held a workshop cosponsored by the journal *Nature* and the Institute of Food Technologists (see discussion of presentation by Tom Neltner, below). This multidisciplinary workshop included more than 60 leading scientists from government, academia, private sector and non-profit organizations from Europe and North America. The take away messages were that the public health implications of not being able to predict adverse health effects at doses relevant to human exposures are significant enough to warrant making the issue a priority, and that there is a need to improve the interdisciplinary communication of endocrinologists, toxicologists and risk assessors to better evaluate these implications.

At a European Commission conference on “Endocrine Disruptors: Current challenges in science and policy” in June 2012 with over 300 participants including policy makers, academics, regulatory risk assessors, industry and NGO groups there was a general recognition by most attendees that the current scientific evidence on risks of EDC to human health and the environment supported the need for action and that the knowledge and tools exist to identify substances with endocrine disrupting properties (http://ec.europa.eu/environment/endocrine/index_en.htm).

Shortly thereafter the European Food Safety Authority (EFSA) held a scientific colloquium with approximately 100 risk assessors and researchers to discuss low dose response in toxicology and risk assessment. Although the different views from different disciplines did not allow for a consensus some pertinent conclusions were noted in the report, (<http://www.efsa.europa.eu/de/events/event/120614.htm>):

1. An adequate and generally accepted definition of “low-dose effects” and of NMDRC is needed in order to facilitate discussions.
2. The amount of evidence needed to decide if in a particular case a “low-dose effect” or an NMDRC has to be taken into account should be defined.
3. Information may be obtained from *in vitro* and *in vivo* studies to determine biological plausibility.
4. Data on toxicokinetics, MoA and toxicodynamics will be helpful to understand the nature of the observations and to link internal dose estimates to occurrence of adverse effects.
5. The criteria for adversity should be the same for all types of effects.
6. It should be possible to derive Points of Departure (PoDs, NOAEL/BMDL) for risk assessment in studies with an adequate (extended range) number of dose levels, in particular in the lower dose range and even if there is a NMDR.
7. Information should be obtained from well-designed studies covering wide dose ranges with more than usual dose groups and sufficient animals per group.
8. Dose selection may be based on observations in epidemiological studies or on estimates of human exposure to cover the low exposure ranges more adequately.
9. It was noted that although the established principles of toxicological risk assessment would still be applicable, adaptation of these techniques might be needed.
10. It was generally considered that tiered approaches for hazard assessment guided by exposure estimates might not be adequate for substances for which an NMDRC is suspected.

Overall, participants considered that the existing risk assessment paradigm is applicable to assess risks that could be associated with low dose/non-monotonic responses. Some participants stated that NMDRC should not be disregarded in risk assessment, whereas others underscored the necessity to understand the mode of action before drawing conclusions for risk assessment.

Thus, implementation of “low-dose effects” and NMDRCs in risk assessment strategies presents a scientific challenge and development of intelligent testing strategies to deal with these phenomena is necessary.

It was clear that different views on the significance of “low-dose effects” and NMDRCs might circulate in different scientific disciplines. Assuming that low-dose effects and NMDRCs are to be accepted as a “fact-of-life”, it should be decided whether these are applicable for specific MoA, or whether they are universal principles applicable to any MoA.

From the discussions, it became clear that there is a need for an in-depth analysis of available studies in which these phenomena have been reported. It was recommended that as a follow-up, EFSA should consider to set up an *ad hoc* multidisciplinary working group to examine the scientific evidence for “low-dose effects” and NMDRCs, and for which MoAs they are applicable.” See discussion of presentation by Iona Pratt, below and the EFSA report (EFSA, 2012).

2. Workshop overview

In order to highlight the recent data on low dose effects and NMDRs for EDCs and to examine data gaps and needs and possible implications of these data for risk assessment a workshop was developed by a planning committee composed of basic scientists, toxicologists, endocrinologists and representatives of regulatory agencies including risk assessors.

The overall goal of the “Low Dose Effects and Non-monotonic Dose Responses for Endocrine Active Chemicals: Science to Practice” Workshop was to determine whether the current data on low dose effects and NMDR for endocrine active compounds (EACs) are sufficient to re-examine the ways in which chemicals are tested for endocrine disrupting properties and how risk to human health should be assessed. This workshop was designed to build off the previous workshops using the Vandenberg et al. review article as a key starting point.

The specific objectives of the workshop are shown in Table 1.

Since the discussion topics were considered to be controversial, the workshop was specifically designed to provide an atmosphere of open, robust and transparent discussion. Key aspects of the design and approach included:

- Open and free registration.
- Planning committee^{***} with representatives from both basic science/academic research backgrounds, and regulatory risk assessment agencies (^{***}Members listed at end of article).
- Consensus not required nor expected.
- Neutral expert facilitators led the discussions in the plenary and breakout sessions to support balanced and respectful discussions.
- Participants were given the option to ask questions anonymously, and reports from break-out groups contained no individual attribution.
- Polling devices used throughout the workshop provided real-time information as to participant opinions to specific questions. The poll answers were anonymous; each participant received a random device with a serial number that he/she kept for 3 d and the responses were tracked by serial number only. This method enabled organizer to:

- Refine discussion group sizes and topics.
- Get feedback from all participants on their analysis.
- Report participant feedback to specific questions presented with demographic information.
- There were two rounds of breakout sessions:
 - The first one was a brainstorming session to cultivate and share opinions and perspectives among participants with like-minded ideas, self-selected by polling.
 - In the second break out, groups were reconstituted to bring together people with a range of perspectives to discuss specific aspects and to refine their ideas, capture the range of views on key issues, identify areas of general agreement and develop a plan for next steps.

Another unique characteristic of the workshop was the development of a “general hypothesis” by the planning committee. This concept, relying on internationally acknowledged key terms, was used to provide the framework for discussion. It also allowed polling of participants on their stance on specific issues. The general hypothesis and definitions are noted in the [supplementary information](#).

The complete workshop program including PDFs of the presentations can be found at

http://ihcp.jrc.ec.europa.eu/our_activities/food-cons-prod/endocrine_disruptors/presentations-workshop-low-dose-effects-endocrine-active-chemicals.

In addition to lectures, breakout sessions, and reports from prior meetings, there were updates from:

- *U.S. Environmental Protection Agency*: Mark Miller introduced EPA’s Non-monotonic Dose Response Curve (NMDRC) Work plan with the goal of publishing the agency Position Paper after addressing the following questions:
 - Do non-monotonic dose response curves (NMDRC) exist for chemicals and if so under what conditions do they occur?
 - Do NMDRCs capture adverse effects that are not captured using our current chemical testing strategies?
 - Do NMDRCs provide key information that would alter EPA’s current weight of evidence conclusions and risk assessment determinations, either qualitatively or quantitatively?
- *Endocrine Society*: Ana Soto summarized the society’s Statement of Principles on Endocrine-disrupting Chemicals and Public Health Protection published in Endocrinology in June 2012 (Zoeller et al., 2012). She emphasized the society’s proposal of using principles of endocrinology to identify and manage EDC risk and the potential irreversible damage caused by exposure to low doses of EDCs during developmental stages.
- *ANSES France*: Claire Beausoleil presented a work ongoing at ANSES on NMDRCs in the field of endocrine disruption. The plausibility of non-monotonicity was assessed using two criteria: statistical plausibility and biological plausibility. These criteria of analysis were developed, based on those initially developed for hormesis, and the biological mechanisms that may be involved were discussed. Fifty-one experimental studies were selected, involving several substances and various effects. Criteria of analysis were applied on 148 NMDRCs. About 55% of them were considered to have a “moderate” to a “high” level of statistical plausibility. Some biases, like cytotoxicity, were discussed. It was highlighted that before considering non-monotonicity in risk assessment, the quality of the NMDRCs has to be assessed. A methodology as well as a decision tree was proposed for testing their plausibility.
- *National Toxicology Program*: Kembra Howdeshell presented a summary of the BPA consortium (called CLARITY-BPA). This is a project supported by NIEHS extramural funding to academic scientists, the National Toxicology Program at NIEHS and the

Table 1

Workshop objectives.

1. To present and discuss the state of the science for low dose effects and non-monotonic dose response curves for chemicals with endocrine activity
2. To define research needs required to move closer to scientific agreement on low dose effects and non-monotonic dose responses for endocrine active substances
3. To define how research programs, especially those funded by public agencies, could support studies that would produce data most useful for a science-based risk assessment
4. To start a discussion on the implications of low dose effects and non-monotonic dose response curves to risk assessment
5. To develop a plan to continue discussions between basic researchers and risk assessment scientists beyond the current workshop

FDA aimed at expanding a guideline toxicity study by adding additional doses (total of six plus two positive controls) and disease focused endpoints assessed blindly by academic researchers who had previously shown these endpoints in their publications (Birnbaum et al., 2012).

3. Setting the stage

Fred vom Saal and Scott Belcher focused on the principles of endocrinology and pharmacology, respectively. The principles of endocrinology establish that hormones:

- Coordinate the development and function of tissues in a highly integrated manner.
- Act via receptors, which are specific, sensitive, and amplify the response.
- Bind to the receptors which are most sensitive to the hormones at the low end of the dose response curve.
- Have affinity for receptors which is distinct from overall *in vivo* potency.
- Produce effects at very low concentrations, in the ppt–ppb range.
- Bind to different receptors as dose increases, resulting in responses not seen at low doses.
- Often result in non-monotonic dose responses due to multiple mechanisms.
- Produce effects that are life stage dependent, with permanent effects occurring following activity during development.

Vom Saal also noted that fetuses lack systemic feedback mechanisms that are present and important in adults. He proposed that the principles of endocrinology should be applied when testing chemicals for endocrine disrupting activity because many EDCs act via receptors, suggesting that EDCs may also show low dose and tissue specific effects, possibly irreversible during development and should be expected to show NMDR.

Scott Belcher noted that examples of NMDR are well accepted in pharmacology for both therapeutic and toxic actions of natural and synthetic compounds including essential nutrients. However, in toxicology, similar NMDR are sometimes considered “spurious” or not “dose responsive” or not “toxicologically relevant”. He also discussed the theory of receptor occupancy and effects; the complexity of receptor subtypes; the effects of co-regulators on hormone response elements; how different ligands could result in different effects via the same receptor; and how positive and negative feedback loops may be responsible for NMDR.

Theo Vermeire gave an overview of the principles of risk assessment. He noted that there is a specific framework for risk assessment which focuses on hazard and exposure assessments while paying attention to uncertainty. Data quality is an important part of the evaluation process and consists of validity of the methods, reliability of test results, consistency and reproducibility of the effects and relevance. Example criteria were discussed briefly. The relevance of observations for humans should be evaluated based on the postulated mode of action. Regarding adversity of effects he noted that risk assessors discriminate between adversity and adaptive or transient effects. When considering the mode of action

of a substance there is a need to establish a causal and possibly dose/concentration relation between *in vitro/in vivo* mechanistic biomarkers and the adverse apical endpoint. Dose response data from all reliable and relevant studies are used to decide on mode of action and the leading health effects. He acknowledged that the current risk assessment paradigm is based on the assumption of monotonicity. Finally, uncertainty and variability regarding duration of exposure, intraspecies sensitivity and interspecies toxicodynamic and toxicokinetic differences are assessed.

4. Main topics arising from breakout sessions

Although a variety of issues and ideas were discussed, they can be grouped into six topics: general hypothesis, low dose, NMDRs, experimental design, risk assessment and weight of evidence, and steps to move forward. Instead of presenting a session by session report of the workshop this overview will focus on the six important topics that were discussed and integrate the data on these topics together. Below we summarize:

- The presentations associated with each of the issues.
- Polling results.

The summary of the breakout discussions reported out to the entire group were formatted into tables as noted below.

4.1. General hypothesis

The general hypothesis stated:

In the context of risk/safety assessments for food, consumer products, and environmental exposures:

- (1) Endocrine disruptors may cause adverse effects at levels relevant to human or environmental exposures.
- (2) These effects may, in some cases, be represented by non-monotonic dose response curves.
- (3) These curves are common enough to warrant formal consideration in risk/safety assessments.

On day 1, the participants were asked whether they could accept the general hypothesis and the three definitions (described in [Supplemental materials](#)) as a starting point for discussions in the workshop. Almost 50% responded that they agreed with them and support their use; 42% said they do not necessarily agree with some or all of the hypothesis but agreed to support its use as a starting point for discussion, and about 9% did not agree with the hypothesis and would not support it as a starting point for discussion.

On the last day, participants were polled again ([Fig. 1](#)). Because of concerns that the hypothesis was ambiguous, the multiple choice answers were modified. Polls showed that:

- 33% Agreed with the hypothesis.
- 47% Could agree if terms were defined or with some minor adjustments to the language.
- 12% Could possibly agree but not without major changes.
- 8% Did not agree.

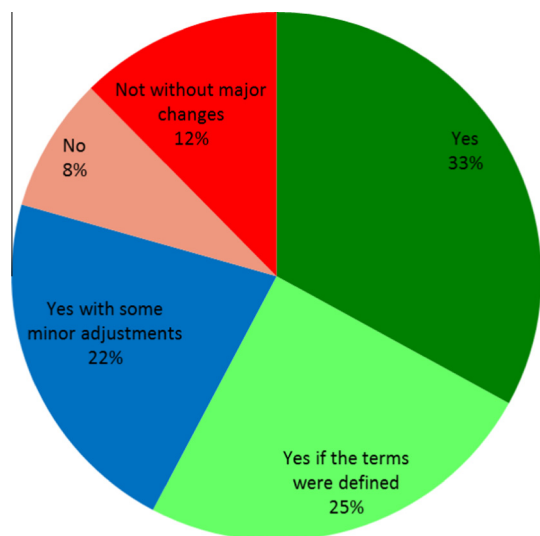


Fig. 1. Polling results at conclusion of meeting. Question: Do you agree with the general hypothesis? In the context of risk/safety assessments for food, consumer products, and environmental exposures: (1) endocrine disruptors may cause adverse effects at levels relevant to human or environmental exposures; (2) these effects may, in some cases, be represented by non-monotonic dose response curves; and (3) these curves are common enough to warrant formal consideration in risk/safety assessments.

Demographics of these results can be found in the [Supplemental material](#).

4.2. Breakout sessions

There were two breakout groups on day 2 consisting of participants that supported the general hypothesis and brainstormed around the topics of how to refine risk assessment or how to overhaul risk assessment. Eighty-five percent of the participants participated in these breakout groups.

The refine risk assessment brainstorming breakout included participants who based on polling results accepted or accepted for the purposes of the discussion that adverse effects at low doses may be common enough to warrant consideration but think that the risk assessment paradigm only needs to be refined to accommodate the findings. They were charged with identifying specific changes to current risk assessment methods with a focus on identifying low dose effects and NMDR.

The overhaul risk assessment brainstorming breakout included participants who based on polling results accepted or accepted for the purposes of discussion that adverse effects at low doses may be common enough to warrant consideration but think that the risk assessment paradigm needs to be substantially changed to respond to NMDRs at low doses. They were charged with explaining why the current risk assessment methodology must be overhauled rather than refined and identifying specific changes needed to each of the four steps in a risk assessment.

These breakout sessions had a robust and open discussion of the issues. Since these were brainstorming sessions the results are not presented here but ideas generated were used by the participants during the day three breakout sessions.

The smaller percentage that did not support the hypothesis as a starting point for discussion (15 participants) were assigned to a third breakout group and charged to:

- Identify one or more specific changes to the general hypothesis and the three definitions and explain the rationale for the changes.

- Describe research studies that would effectively test the new hypothesis based on the changes proposed.

These 15 participants concluded that the general hypothesis was comprised of three different hypotheses and that each was flawed, also indicating that the first item lacked precise language, the second stated nothing new, and the third used the term “common enough” which was too general.

This group did not develop an alternative hypothesis. Instead they agreed that “refinement of current toxicological methods should adequately account for endocrine disruption.” They called for “evolution not revolution” and said that research studies should “target efforts on evaluating current testing methodology and making necessary refinements.”

On day 3 five breakout groups were organized, one on data gaps, two on experimental design issues and two on risk assessment methodology. Participants were assigned to the breakout groups based on polling results and the goal of ensuring adverse mix of perspectives and keeping the number of participants equal in each session. The questions asked in the breakout sessions can be found in the [Supplemental material](#). The responses to questions related to these breakouts can be found in [Tables 2–7](#).

4.3. Low dose effects

Tyrone Hayes introduced low dose effects for EACs (the terms EACs and EDCs were not formally differentially defined and were used interchangeably throughout the workshop), defined as either effects occurring at doses lower than those typically used in standard testing protocols, or in the range of human exposure. He reported that, in addition to the four EDCs described in the 2002 NTP report, low dose effects have been shown for another 24 EDCs (Vandenberg et al., 2012). He stated that this low number may be due to the fact that the majority of EACs have not been tested at “low doses”. He detailed weight of evidence analysis for atrazine and BPA developed using principles of endocrinology. Evidence for low dose effects was given higher weight when there were multiple studies, that were performed in multiple labs; additional considerations included whether the experimental model was responsive to low doses of hormones, the system was free from contamination and positive and negative controls were used. He concluded that low dose effects should be expected for EDCs because they follow the same “rules” as hormones.

The low dose issue was discussed in several breakout sessions on day three. [Table 2](#) shows the results of discussions in breakouts related to experimental design issues related to low dose effects. Data gaps and needs in the area of low dose effects and NMDR identified in breakout sessions are noted in [Tables 3–5](#) and possible steps over the next 5 years to address low dose effects and NMDR are noted in [Table 6](#).

[Fig. 2A](#) shows a summary of the polling results related to low dose effects. An additional question was also asked:

Question: the definition of low dose should be:

- Human exposure range and/or environmental levels: 41%.
- Any dose at or below the NOAEL of the most sensitive species tested: 18%.
- NOAEL divided by an uncertainty factor (which could be flexible or fixed): 7%.
- No formal definition is needed, just state what you mean: 22%.
- Other: 12%.

4.4. Non-monotonic dose response issues

Laura Vandenberg described the state of the science for NMDR for EDCs. She started by noting that

- (1) Non-monotonicity is defined mathematically as a curve that changes slope from one direction to another and therefore can occur in any part of the dose response curve, not only at low doses.
- (2) Natural hormones show NMDR but not necessarily for every endpoint and for all tissues. She showed examples of NMDR from guideline studies and epidemiology studies, while providing more than 30 examples of chemicals showing NMDR *in vitro* and *in vivo*.

Vandenberg also discussed whether the endpoints showing NMDR are adverse; and indicated that, while some of the endpoints noted would be considered adverse in guideline studies, most would be considered adverse by endocrinologists. She highlighted that not all regulatory agencies have a definition of adverse effect; therefore the determination of adversity often relies on expert judgment. She ended by acknowledging the need for NMDR reproducibility and underscoring that the occurrence of NMDR challenges the idea that high doses can predict the effects of chemicals at lower doses.

Rory Connolly discussed computational modeling of NMDR, and started by indicating that a study with only three doses covering a high dose range cannot answer the question of whether NMDR exists. He explained different intracellular factors leading to NMDR such as saturation of metabolic pathways, alteration of signaling pathways and induction of detoxification pathways. Additionally, NMDR can arise when xenobiotic exposure elicits at least two effects and when these effects can individually affect a common endpoint, such as simultaneously increasing the number of adducts and their repair mechanism. He concluded that ongoing research on toxicokinetics and exposures to EAC as well as its metabolites

Table 2
Experimental design issues related to low dose effects.^a

- Use sensitive endpoints in sensitive animal model
- Include developmental exposures as a sensitive time for exposures
- Consider lifetime exposure studies
- Use three or more doses
- Analyze experimental design for statistical power
- Measure internal dose of environmental chemical to aid in extrapolation to humans

^a Points taken from breakout group reports, some wording changes for clarity and to avoid overlap.

Table 3
Data gaps and needs in area of low dose effects and NMDR.^a

There is a need for:

- ‘Appropriate’ data on chemicals to judge binding affinity and intrinsic activity at a receptor compared to endogenous hormone
 - At what point is the potency so high that it is of concern relative to the effects that can occur with high affinity and high intrinsic activity?
- Human exposure data for new and existing chemicals
- Environmental (wildlife) exposure data for new and existing chemicals
- To determine if low dose effects are relevant for ecotoxicity at the population level
- Information on internal dose from *in vitro* and *in vivo* experiments in order to be able to translate across species
- Information on reproducibility of experiments and adversity of endpoints measured as they relate to low dose studies
- To include/address issues of variability, reproducibility and information on chemical purity in studies assessing low dose effects and NMDR
- Determination of what fits the definition of adverse effects
- To involve specialized experts from relevant fields to assess toxicological findings
- Information on endocrine mode of action besides estrogen, androgen and thyroid

^a Points taken from breakout group reports, some wording changes for clarity and to avoid overlap.

Table 4
Experimental design issues related to NMDR.^a

- NMDR must be reproducible and consistent across laboratories
 - Consider background contamination (feed, reagents, tissue culture materials)
 - Consider variability due to animal stress, circadian rhythms
 - Reproduce effects within labs before publication
 - Evaluation criteria for assessment of dose responses for non-monotonicity should be included in manuscripts
- Encourage funding and publication of two laboratories with same question/approach/dose range
- Authors should evaluate dose responses for NMDR using statistical analysis and report results as part of discussion in manuscripts

^a Points taken from breakout group reports, some wording changes for clarity and to avoid overlap.

is needed for understanding when such dose–responses are likely to occur under real-world exposure conditions.

Ana Soto noted that NMDRs are common in endocrinology and described seven proposed mechanisms to explain their occurrence as follows and showed examples of each mechanism:

- Cytotoxicity.
- Receptor down-regulation and desensitization.
- Cell and tissue specific receptors and cofactors.
- Receptor selectivity.
- Receptor competition.
- Negative feedback loops.
- Tissue interactions.

She explained that, in many cases, NMDR were due to competing pathways which change over a dose range. For example, an EDC may interact with a specific receptor at low doses and, as the dose increases, it activates other receptors and thus other pathways. Another mechanism is represented by the activation of different pathways by the same receptor at different doses such as in breast tissue where BPA can cause cell proliferation over a large dose response but at higher doses it causes apoptosis. These competing pathways lead to NMDR. She also noted that for many NMDR the mechanisms are unknown but that should not be a determinant in accepting the existence of the phenomenon.

Fig. 2B and C shows a summary of the polling results related to NMDRs.

4.5. Experimental design issues

Olwenn Martin described endocrine endpoints within OECD test guidelines. She noted that the OECD conceptual framework is not a testing strategy but a 5 level toolbox that consists of validated *in vitro* and *in vivo* assays. Regarding endocrine endpoints, OECD test guidelines focus on estrogen, androgen and thyroid effects. For example, the 28 d repeated toxicity study has been updated to include endocrine endpoints mostly focusing on histopathology and organ weight. She mentioned that although critical windows of exposure are included in multigenerational reproductive tests and partial or full lifecycle assays they include limited endocrine relevant endpoints. She stated that guideline studies usually include three dose levels based on toxic effects and available toxicokinetic data and a control with monotonicity assumed. However, she noted that in the 2012 guidance on chronic toxicity and carcinogenicity assays OECD states that there may be non-linearities in the dose response, and that doses should cover anticipated human exposure levels. She ended saying that it would be helpful to extend this guidance to other assays within the conceptual framework and to consider additional endpoints which relate to additional hormonal and disease pathways.

Table 5Experimental design issues related to low dose effects and NMDR in risk assessment.^a

- Add endocrine endpoints that are not formally/currently validated to standardized toxicity tests, for example, mammary glands, glucocorticoids/stress response, diabetes/metabolic disorders, obesogens (ppar gamma pathways)
- Identify new biomarkers that can predict environmental and public health outcomes
- Stimulate investigators to report low dose data, arbitrary findings and negative data
- Practical assessment of what fits the definition of adverse effects would be good/helpful
- Standard and accepted definition of “low dose” is needed
- Separate the issues of low dose effects from that of NMDR
- Adapt/change guideline studies: start dose selection at low doses and look for NMDR
- Participants were evenly split whether a non-threshold model for endocrine disruptors is appropriate
- Risk assessment portfolios should include toxicokinetic data and include better internal dose data
- Build informed cases: more documentation and justification for decisions
- Better access to raw data, especially in published papers
- Establish formal routes of communication, especially between risk assessors and academics providing data. Need transparency and better documentation of the decision making process
- Develop new test methods for step 1 (hazard identification) of risk assessment: assays that reflect endocrine endpoints and critical windows
- Enhance available assays: endocrine endpoints, critical windows (extended 1 generation study may be a good first step)
- Address data gaps in our knowledge of exposures at vulnerable periods (fetal)
- Take into account allometry in administered doses (where appropriate) to choose “low doses”
- Integrate existing classification system with recognized endocrine endpoints
- Establish an agreed upon definition of “low dose” or ask all to be precise in their language
- Develop new test methods
- Recognize that processes need to proceed in parallel, and information needs to be continually integrated
- Studies that show an effect at low dose which disappears at high dose should not be dismissed
- Continue to use a tiered approach to exclude chemicals that are unlikely to cause endocrine disruption
- Use of QSAR and *in vitro* multiple dose screening to have indications for further testing
- Develop or elaborate on guidance which allows evaluation of exploratory research, to make it more useful to Risk Assessment
- Use of assays developed by exploratory research in risk assessment: aid risk assessors to understand and interpret them
- Consider using test methods with consistent results even if not formally validated

^a Points taken from breakout group reports, some wording changes for clarity and to avoid overlap.**Table 6**Possible steps over 5 years to address low dose effects and NMDR.^a

- Develop a knowledge base of findings of *in vivo* and *in vitro* NMDR and low dose effects in a secure database as a resource. This might include raw data
- Develop minimum information requirements for publishing NMDR using a task force of academic, regulatory, and industry laboratories. Use OECD guidelines as a starting point
- Define adverse for purposes of low dose effects and NMDR
- Prioritize and fill the data gaps noted
- Integrate existing classification system with recognized endocrine endpoints
- Establish an agreed upon definition of “low dose” or ask all to be precise in their language
- Develop new QSAR training sets that also work at low doses

^a Points taken from breakout group reports, some wording changes for clarity and to avoid overlap.**Table 7**Steps needed to expand/improve risk assessment over the next 5 years^a.

- Develop and publish further guidance/criteria for increasing the use of exploratory academic studies (including power calculations and sample size) in risk assessment
- Create and publish an evolving list of relevant adverse effects that should be looked at in toxicity studies
- Find a consensus on “low dose” and provide guidance on how and when to add lower doses
- Include MoA and adverse outcome pathways initiative within the low dose area (WHO and OECD)
- Determine to what extent evaluation of data from current study protocols pick up NMDR
- Determine to what extent current testing protocols pick up NMDR
- Build on OECD guidelines to make better use of current data and include additional (low) doses or effects with latency
- Implement the use of power analyses and statistical methods in choice of endpoints (including continuous versus binary), as well as the number of animals needed
- Enhance existing assays for endocrine endpoints and implement new test methods into regulation

^a Points taken from breakout group reports, some wording changes for clarity and to avoid overlap.

Table 4 shows the results of a breakout session on day three that discussed experimental design issues related to NMDR. Additionally, Fig. 3 shows a summary of the polling results related to experimental design.

4.6. Risk assessment and weight of evidence issues

Studies that follow specific testing guidelines from regulatory agencies (known as guideline studies) generate a wealth of data regarding the potential health consequences of exposure to chem-

icals. The assumptions that underlie guideline studies have *a priori* impact not only on experimental design but also data analysis and interpretation.

Heather Patisaul illustrated this point describing two studies that followed OECD guidelines. She showed that in both cases, the statistically significant adverse effects either followed non-linear responses or the effects that were observed only at the lower dose were considered by the authors to be “incidental” or “spurious” and thus “unrelated to treatment.” They were not reported in the study summary or considered when establishing the NOAEL.

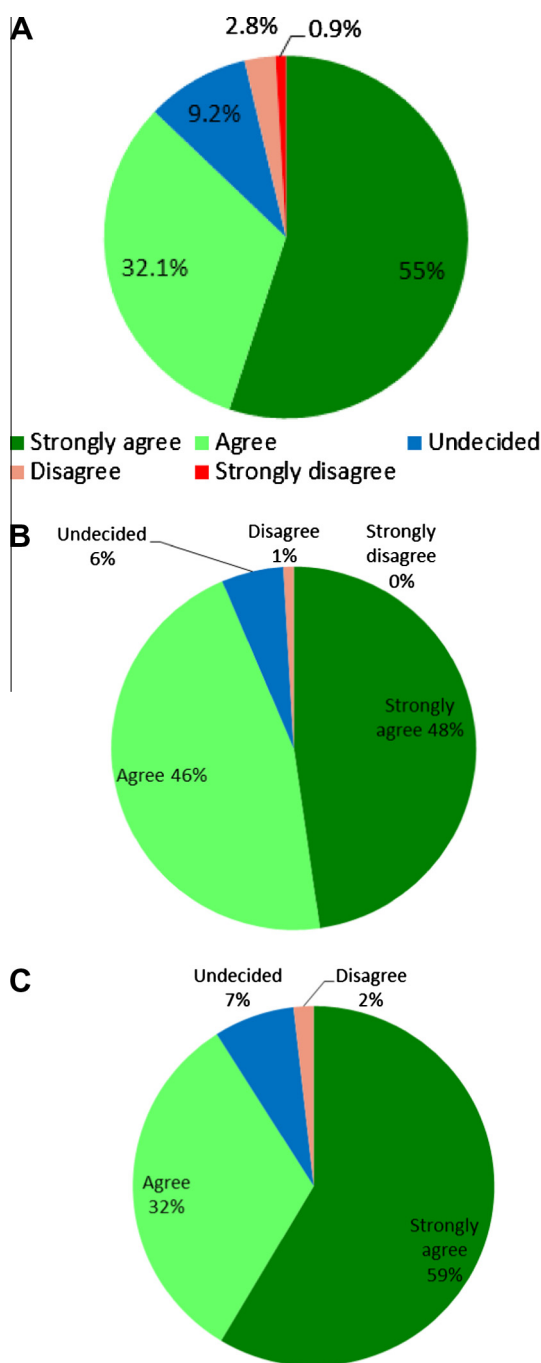


Fig. 2. Summary of polling results related to low dose effects and NMDRs. (A) Question: do you agree that a low dose definition would be helpful/good? (B) Question: Do you agree that NMDRs exist at some dose range? (C) Question: Do you agree that NMDR is a different issue than the low dose issue?

Among the adverse effects were fewer live births, skeletal malformations in the offspring, and altered adrenal, thyroid and pituitary gland weights. Data analysis itself was also impacted by the presumption that effects would be monotonic. For example in a particular developmental toxicity study, numerous endpoints were not statistically analyzed if an effect was not observed at the highest dose.

Patisaul also showed that her own work indicates that perinatal exposure to the same chemical at a dose 10-fold lower than the NOAEL produced statistically significant effects consistent with those observed in the guideline studies, suggesting that the true

NOAEL is lower than what was concluded. She stated that failing to consider that effects might be non-monotonic in guideline studies results in the discounting of data points that might be informative for NOAEL calculation.

Alexandre Feigenbaum presented risk assessment at EFSA. He noted that EFSA takes into account all available information, whether from public organizations or from industry, GLP or non-GLP and targets overall scientific consistency. He used the BPA opinion as an example and pointed out the transparency of the opinion, reminding that criteria set for inclusion of studies in the risk assessment are published in the opinion. He noted that in 2010 in the preparation of its opinion on BPA, the CEF Panel had screened more than 800 scientific studies (EFSA, 2010). These studies were critically reviewed by a large panel of experts and their relevance to public health was carefully assessed. One of the conclusions was that the Panel is “not aware of any clearly reproducible adverse effect expressed specifically at low BPA doses only”; studies claiming for low dose effects did not meet the criteria for inclusion in the risk assessment. He also gave the example of a GLP study provided by industry for that opinion and for which the Panel did not agree with the conclusions. Feigenbaum also pointed out that the experts of EFSA are selected on the basis of their scientific excellence and their independence. He concluded that EFSA is neither for nor against the low-dose hypothesis, as this is still the subject of considerable debate. EFSA is open to any new scientific information and is directly involved in the scientific debate.

As part of risk assessment issues, Jim Bridges discussed the impact of low dose and NMDR on future risk assessments. He indicated that the pros of the current risk assessment procedures are that they are familiar to all stakeholders, resource implications are known, and they have a good record of protection of human health. On the con side, they have a high demand on resources, provide limited information on dose response relationships and have provided a very modest contribution to advancing scientific understanding.

In the current climate there are several reasons for reviewing current risk assessment procedures, according to Bridges. These include:

- Demands to reduce or abolish the use of animals for toxicity testing.
- Recognized deficiencies in current approaches.
- The large number of untested chemicals and chemical products.
- The need to better assess effects of exposures to combinations of chemicals.
- Advances in science and challenges posed by more complex products such as nanotechnologies.

Bridges said that all stakeholders have an interest in modifying current risk assessment. He showed a list of possible priorities which included characterizing the range of human exposures, determining critical windows for exposure relevant to man, identifying vulnerable groups, understanding the impact of exposure to multiple stressors and examining effects over a much greater dose range. He noted that future risk assessments can be anticipated to have better exposure measurements, more reliance on *in vitro* and *in silico* methods, increased use of “omics” endpoints enabling earlier detection and improved sensitivity, and a progressive change to a mode-of-action basis for evaluating hazardous substances. With regard to dose response he asked whether high dose effects are relevant to exposures at lower doses; is the methodology used to set NOAELs sufficiently rigorous; and are we missing effects of toxicological concern by not assessing low dose effects and NMDR? He ended his presentation with a challenge: *More than at any other time risk assessment is at a crossroads one path leads to*

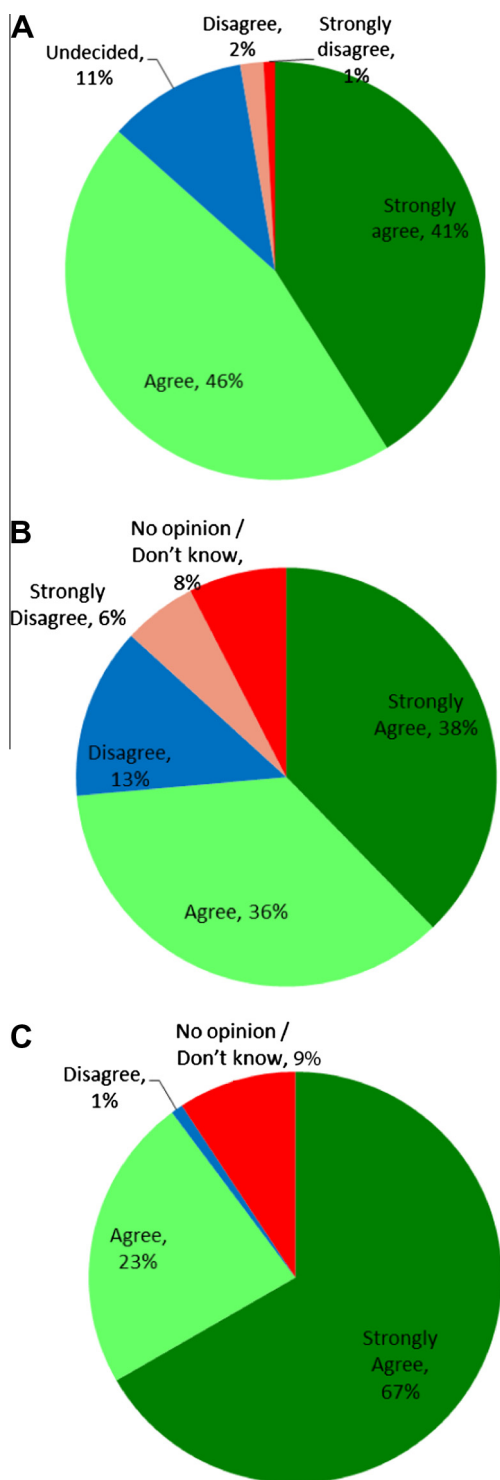


Fig. 3. Summary of polling results related to experimental design. (A) Question: Do you agree that a practical assessment of what fits the definition of adverse effects would be helpful/good? (B) Question: I could support the concept of adding ED endpoints that are not formally/currently validated to standardized tests. (C) Question: I would support the development of a list of desirable information to be included in a peer reviewed publication so it may be useful for risk assessment.

retaining the status quo the other to a challenging but less certain future. Let's hope we have the wisdom to choose correctly.

Fig. 4 shows a summary of the polling results which focused on risk assessment and weight of evidence. An additional question was also asked:

Question: what is the best way of incorporating academic data into the risk assessment process?

- 63%: Develop new criteria specifically for this area research to evaluate quality.
- 28%: Use the same criteria currently used to evaluating guideline studies to evaluate the quality of academic studies.
- 8%: The system is fine the way it is.
- 1%: Academic data should not be used in risk assessment.

5. Steps to move forward

The goal of the workshop was to openly discuss the hypothesis proposed and to, based on the discussion, either accept the hypothesis as is or determine what further research would be needed to accept the hypothesis and in either case determine how acceptance of the hypothesis would impact risk assessment. The one topic where there was consensus is that more is needed to be done in the area of understanding the importance and impact of low dose effects and non-monotonic dose responses to EDCs and changes needed to risk assessments to accommodate these effects: including more workshops, more literature reviews and improved communication between relevant parties. Breakout groups were asked to propose ideas to move the discussion forward over the next 5 years. Table 3 shows data gaps noted, Table 6 shows some of the ideas discussed related to addressing low dose effects and NMDR and Table 7 shows ideas presented relevant to expanding or improving risk assessment.

Many of the breakout group recommendations would be facilitated by and may even require more formal routes of communication, especially between risk assessors and academics providing data. A series of workshops and/or task forces of academic, regulatory and industry scientists to develop such guidance for publication was suggested as one possible means of moving forward the "low dose" hypothesis as developed at this workshop. These workshops would focus on the following:

- Development of guidance documents useful to both researchers and risk assessors.
- In depth analysis to determine to what extent current study protocols pick up NMDR and to what extent evaluation of study protocols may overlook NMDR because of the default assumption of monotonicity.
- There is a need for some guidance on minimum information requirements for reporting non-guideline studies by researchers so the data would be more useful for risk assessors, as well as guidance on how risk assessors could use these studies in their assessments. This might be best accomplished by a working group that would develop guidance for publication.
- There was also a call from the research community for transparency and better documentation and more justification of the decision making process in risk assessment, this could be facilitated by the availability of raw data not only from research studies but also regulatory guideline studies.
- A workshop to define additional endpoints and how to facilitate their incorporation into guideline studies is needed to improve the sensitivity of current guideline studies to identify endocrine disrupting substances as done for the recently enhanced extended one generation reproduction study. Consideration of additional biomarkers related to human diseases of increasing incidence, such as diabetes and breast cancer and to cover more aspects of the endocrine system beyond estrogen, androgen and thyroid hormone pathways was also proposed, for example, effects on mammary glands, glucocorticoids (in relation to stress responses), and PPAR γ pathways (in relation to fatty acid metabolism/obesity and metabolic disorders).

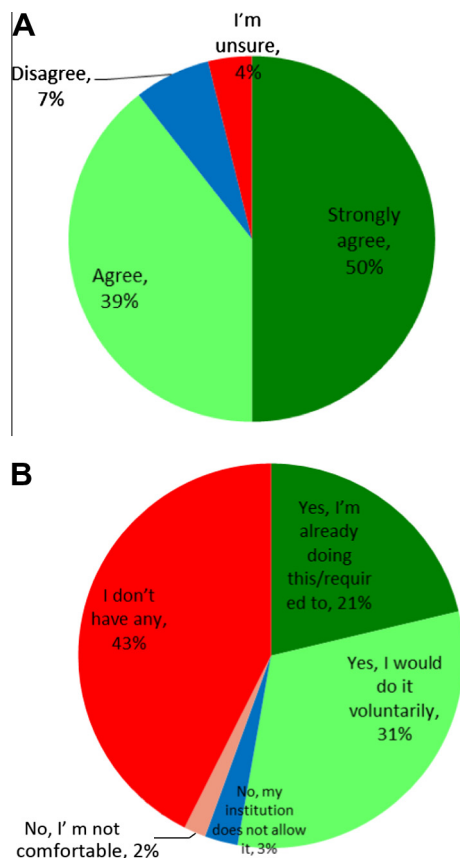


Fig. 4. Summary of polling results related to risk assessment and weight of evidence. (A) Question: Principles and procedures need to be developed to promote a more systematic use of all available data in the risk assessment process (including non-guideline, epidemiological and biomonitoring data, etc.). (B) Question: Do you agree to provide your raw data on request by regulators/risk assessors?

- A workshop to develop a consensus on the definition of low dose and a consensus on the significance of the 'low dose' issue followed by guidance on how and when to include 'low doses' in the design of toxicology studies, considering the need for not only adding more doses to testing protocols but to start dose selection at "low doses" rather than from high doses downward.
- A knowledge base of findings *in vivo* and *in vitro* showing NMDR and low dose effects in a secure database as a resource, which may include raw data, was also proposed.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.chemosphere.2013.06.043>.

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